Novel roles of Top3 and Rmi1,members of the Sgs1-Top3-Rmi1 complex,in establishment of sister chromatid cohesion(姉妹染色分体の接着におけるTop3とRmi1の新たな機能の解析)

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学位記番号	薬　博　第　432　号
学位授与年月日	平成 21 年 3 月 25 日
学位授与の要件	学位規則第 4 条第 1 項該当
研究科、専攻	東北大学大学院薬学研究科 （博士課程）生命薬学専攻

学位論文題目

Novel roles of Top3 and Rmi1,members of the Sgs1-Top3-Rmi1 complex, in establishment of sister chromatid cohesion
（姉妹染色分体の接着における Top3 と Rmi1 の新たな機能の解析）

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論 文 内 容 要 旨

【Background】

Bloom syndrome (BS) is a rare autosomal recessive disorder characterized by severe growth retardation, immunodeficiency, reduced fertility and predisposition to various types of cancers. BS is caused by mutation in the BLM gene, which encodes a DNA helicase of the RecQ family. BLM associates with TOPOIIIα(DNA topoisomerase IIIα) and RMI1 (RecQ-mediated genome instability) to form an evolutionary conserved complex that functions coordinately to process a diverse array of DNA structures. RecQ ortholog in budding yeast Sgs1 also interacts with Top3 and Rmi1. Sgs1-Top3-Rmi1 complex is essential in the resolution of recombination intermediates, restarting stalled replication forks, damage-induced recombination repair and activation of DNA replication checkpoint response.

Because of the relatively recent discovery of Rmi1, the understanding of the cellular roles and contributions of Rmi1 to Sgs1-Top3 complex still poorly understood and remain untested. In this study, I aim to elucidate the molecular functions of Rmi1 and biological processes in which Rmi1 is involved, using budding yeast as a model organism. Here I show novel roles of Top3 and Rmi1 in establishment of sister chromatid cohesion, a process that keeps the two copies of sister chromatids together from the moment of duplication to the onset of anaphase.

【Results】

1. Mitotic defects of *rmi1* mutants

Previous work has shown that cells lacking *RMI1* show slow growth and a large-budded morphology. To determine whether the slow growth phenotype of *rmi1* cells was accompanied by a specific defect in cell cycle progression, I examined the cells by flow cytometry. Consistent with previous reports, I found that *rmi1* cells remained blocked in G2/M phase when compared with wild type cells. Microscopic analysis of asynchronous cultures of *rmi1* cells showed an increase in the percentage of large-budded cells and cells with intermediate-length spindles, morphology characteristics of G2/M phase. In addition, a marked increase in the number of cells with aberrant spindle structures was observed in *rmi1* cell population.

Generally, mitotic delays could reflect activation of either the DNA damage checkpoint or the mitotic spindle checkpoint. Previous work reported that the Rad53-dependent DNA damage checkpoint is activated in *rmi1* cells. Therefore I examined whether the mitotic spindle checkpoint is activated as well in *rmi1* cells. Interestingly, I found that the G2/M accumulation of *rmi1* cells was partly suppressed by deletion of *MAD2*. I also observed that the anaphase inhibitor Pds1 was stable in *rmi1* cells. These results indicate that the absence of *RMI1* activates the Mad2-spindle checkpoint, resulting in a delay in the progression of M phase. Furthermore, I found that *rmi1* cells were moderately sensitive to benomyl, a microtubule depolymerizing drug, indicating that Rmi1 may involve in some aspect of chromosome segregation.

2. Defective sister chromatid cohesion in *rmi1* and *top3* mutants

It has been shown that *ctf7* and *ctf18* mutant cells, which have defects in sister chromatid cohesion, show Mad2-dependent M phase arrest and have intermediate-length spindles. As *rmi1* cells showed a similar phenotype to that of *ctf7* and *ctf18* mutants, I tested whether Rmi1 has a role in sister chromatid cohesion. Chromosomal cohesion ensures the accurate segregation of only one copy of each chromosome to each daughter cell. It is mediated by the conserved essential cohesin ring complex, that in budding yeast consists of Smc1, Smc3, Scc1 and Scc3.

I first assessed the genetic interactions between *RMI1* and cohesion-related genes. I found that *RMI1* genetically interacts with *SMC1*, the cohesin subunit, suggesting that Rmi1 might involve in sister chromatid cohesion. I then assessed cohesion directly by using strains that contain Tet operator repeats and can express Tet repressor-GFP fusion protein. Intriguingly, I found that *rmi1* cells exhibited an increase in the number of two GFP signal foci when compared with wild type cells, indicating a defect in sister chromatid cohesion. Rmi1 interacts physically and functionally with Sgs1 and Top3. I next examined whether Sgs1 or Top3 was also involved, along with Rmi1, in sister chromatid cohesion. Unexpectedly, I found that *top3*, but not *sgs1* mutant cells, were also defective in sister chromatid cohesion.

Most of the defects shown by *top3* and *rmi1* cells are suppressed by mutation of *SGS1*. I next tested whether this is also the case for cohesion defects. I found that *SGS1* deletion could suppress the cohesion defects in both *top3* and *rmi1* cells. In addition, the cohesion defects in *rmi1* cells was considerably suppressed by *RAD51* mutation. These results indicate the existence of a new pathway involving Rad51 and Sgs1-Top3-Rmi1, which leads to the establishment of sister chromatid cohesion.

3. Molecular mechanism of Rmi1 in sister chromatid cohesion

I found that *rmi1* cells showed severe growth defect when the cohesion establishment factor Eco1 was impaired, suggesting a possible function of Rmi1 in cohesion establishment. It was reported that proteins required for sister chromatid cohesion act close to the replication forks. Using chromatin immunoprecipitation assay, I confirmed that Rmi1 were enriched at an early-firing replication origin ARS607, at least when cells were arrested in early S phase by hydroxyurea treatment. Furthermore, I found no significant difference in cohesin binding between wild type and *rmi1* cells under the same condition, suggesting that loss of Rmi1 does not hamper cohesin loading.

Nonessential genes such as *CTF18*, *CTF8*, *DCC1* and *CTF4* are found to make critical redundant contributions to the establishment of sister chromatid cohesion. The replication factor C (RFC)-like complex Ctf18-Ctf8-Dcc1 and Ctf4 have a mutually distinct role in sister chromatid cohesion. I found that Ctf4, but not Ctf18 binding at the replication forks, was affected in the absence of Rmi1, suggesting that lack of Rmi1 leads to sister chromatid cohesion defects through reduced function of Ctf4. Unexpectedly, using genetic approach, I found that Rmi1 functions in the same cohesion pathway as the Ctf18-RFC complex, but not Ctf4. These results indicate that lack of Rmi1 might perturb Ctf18 function around DNA replication fork.

4. Mus81 in sister chromatid cohesion

I aim to identify additional genes to be related to Rmi1 with roles in sister chromatid cohesion. Since *rmi1* cells were lethal when combined with *mus81* mutants, I examined the status of sister chromatid cohesion in *mus81* cells. As expected, I observed a cohesion defect in *mus81* cells. Because Rmi1 and Mus81 are proteins required for replication fork integrity, their roles in sister chromatid cohesion may provide a genome-wide protection to the chromosome integrity.

【Conclusions】

Since Sgs1-Top3-Rmi1 complex plays a role in processing homologous recombination intermediates, Rmi1 may promote the binding of Top3 specifically to branched DNA structures and/or its strand passage activity. Interpreting the results above, I hypothesize that Sgs1 generate recombination intermediates that require Top3 and Rmi1 for processing to establish proper sister chromatid cohesion. In the absence of Rmi1, unprocessed and/or aberrantly processed homologous recombination intermediates may at least partly contribute to the cohesion defects observed.

The molecular mechanism of Rmi1 in sister chromatid cohesion still remains ambiguous. One thought is that Rmi1 binds at replication forks during S phase and may stabilize other replisome components such as Ctf4. This allows correct architecture of the replication forks to pass through the cohesin ring without disrupting it. Rmi1 may also function to prevent irregular decatenation activity of Top3. Together with other nonessential cohesion genes like Ctf18-RFC complex, Rmi1 may likely act in redundant pathways to facilitate proper establishment of sister chromatid cohesion.

Very recently, synthetic lethal genes with *rmi1* mutation, *RRM3* and *ESC2*, when both genes are disrupted, reported to be defective in sister chromatid cohesion. Taken together my findings of Rmi1 and Mus81 with those of Rrm3 and Esc2, it suggests that preservation of replication fork integrity is generally a pre-requisite for proper establishment of sister chromatid cohesion.

審 査 結 果 の 要 旨

RecQ ファミリーヘリカーゼはゲノムの安定維持に重要な役割を果たしており，ヒトでは，この RecQ ファミリーヘリカーゼの機能の欠損が，がん化や早期老化の原因になることが知られている。本研究は，RecQ ファミリーヘリカーゼの機能の欠損とがん化や早期老化との関係を分子レベルで解析するために，モデル生物の出芽酵母を用い，出芽酵母 RecQ ヘリカーゼである Sgs1 と複合体を形成する DNA トポイソメラーゼ III（Top3）と Rmi1 のうち特に Rmi1（RecQ-mediated genome instability 1）の機能に注目して解析を行ったものである。

まず初めに，この *RMI1* 遺伝子の変異株を作製し，その性状解析を行った。その結果，*RMI1* 変異株は増殖が遅く，G2/M 期に停止する傾向がみられ，微小管を脱重合させるベノミルに感受性であることが判明した。一方，spindle checkpoint に関与する *MAD2* 遺伝子を破壊すると G2/M 期の蓄積が抑制された。このような表現型はコヒージョン（姉妹染色分体の接着）に欠陥のある変異株で観察されることから，コヒージョンに欠陥があるかどうかを調べ，*RMI1* 変異株はコヒージョンに関与する *CTF18* の変異株と同程度の欠損を示し，Rmi1 がコヒージョンに関与するという予想外の発見をした。

次に，*SGS1* 変異株と *TOP3* 変異株で，コヒージョンの欠損を調べたところ，*TOP3* 変異株はコヒージョンの欠損を示すが *SGS1* 変異株は欠損を示さなかった。一方，*RMI1* 変異株，*TOP3* 変異株のコヒージョンの欠損は *SGS1* 遺伝子あるいは *RAD51* 遺伝子の破壊で抑制された。以上の結果から，Sgs1-Top3-Rmi1 複合体は Rad51 により形成されたヘミカテナン様の構造を解消するとともに，Top3-Rmi1 は分離した姉妹染色体のコヒージョンに関与する可能性が示唆された。

近年，コヒージョンの確立に関わる 2 つの経路の存在が明らかにされた。それは，Ctf18，Ctf8，Dcc1 及び Mrc1 が関与する経路と，Ctf4，Chl1，Csm3 及び Tof1 が関与する経路である。最後に，Top3-Rmi1 がこれらの経路とは異なる新規な経路でコヒージョンの確立に関わるのか，それともこれらの経路のうちのどちらで機能するのかを，二重遺伝子変異株を作成して遺伝学的に解析した。その結果，Top3-Rmi1 は Ctf18 や Ctf8 が関与する経路で機能することが明らかになった。

以上，本研究は，Top3-Rmi1 複合体と Sgs1 の今まで知られていなかった新規な機能を明らかにしたもので，Sgs1 は高発癌性で知られるブルーム症候群の原因遺伝子産物 BLM に対応し，ブルーム症候群のゲノム不安定性や高発癌性の分子機構に迫るもので，当該分野の研究の進展に大きく寄与するものである。

よって，本論文は博士（薬学）の学位論文として合格と認める。